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Nitrogen accumulation, rather than carbon: nitrogen
stoichiometric variation, underlies carbon storage during forest
successionJunliang Zou¹ , Juying Wu¹, Bruce Osborne², Brian Tobin³ and Yiqi Luo⁴¹ Beijing Research & Development Centre for Grass and Environment, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, People's Republic of China² UCD School of Biology and Environmental Science and UCD Earth Institute, University College Dublin, Belfield, Dublin 4, Ireland³ UCD Forestry, School of Agriculture and Food Science and UCD Earth Institute, University College Dublin, Belfield, Dublin 4, Ireland⁴ Center for Ecosystem Science and Society, Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ, United States of AmericaE-mail: jlzou@nwafu.edu.cn and wujuying@grass-env.com**Keywords:** carbon sink, carbon–nitrogen coupling, forest succession, nitrogen limitation, stand age, stoichiometrySupplementary material for this article is available [online](#)**Abstract**

Forest ecosystems play an important role in regulating global temperatures through their capability to take up and store CO₂ from the atmosphere, but the magnitude and sustainability of this carbon (C) sink is critically dependent on the availability of nutrients, particularly nitrogen (N). However, the extent to which the absolute amount of N or modifications in plant and soil C:N ratios controls long-term forest carbon sequestration (C_{seq}) remains uncertain. To assess this, we analyzed the results of 135 global field studies that investigated the dynamics of C and N availability during forest succession. The results showed that the accumulation of C and N in plant (including above- and below-ground vegetation) and litter pools decreased with forest age and approached an equilibrium value in the latter stages of stand development. Plant and litter C:N ratios increased during the first 10–20 years and remained relatively stable thereafter. The analysis further showed that the relative importance of a change in the total amount of N or modifications in the C:N ratio, to increases in C_{seq}, varied with forest age. Whilst the relative importance of a change in the total amount of N increased with forest age, the relative importance of a varied C:N stoichiometry decreased with forest age. Overall, a change in the total amount of N was the more important factor contributing to C storage during forest stand development and the C stored in vegetation dominated the total ecosystem C pool. These results show that ecosystem N availability is a key factor supporting long-term forest C_{seq} during forest succession. As most of the C is found in above-ground vegetation, this pool is particularly susceptible to abiotic or biotic factors and anthropogenically-related disturbances.

1. Introduction

Large scale anthropogenic activities, such as deforestation, land use conversion, and fossil fuel combustion have led to increased atmospheric carbon dioxide (CO₂) concentrations, which has accelerated global warming (Stocker *et al* 2013). In response to this, there have been many proposals about how atmospheric CO₂ concentrations might best be reduced and/or how carbon sequestration (C_{seq}) might be

enhanced. Secondary forests are a globally important terrestrial carbon (C) sink (Kauppi *et al* 2006), and tree planting provides a potentially effective way to remove atmospheric CO₂ (Houghton *et al* 2009). Young forests have been shown to store large amounts of C in tree trunks, roots and soil over relatively short time periods due to their rapid regeneration rate (Chazdon *et al* 2016). However, whether this large capacity for C_{seq} can be maintained during the later stages of forest succession remains unclear.

Growing evidence suggests that nitrogen (N) availability can regulate C fixation by terrestrial ecosystems (Rastetter *et al* 1997, Finzi *et al* 2001, Hungate *et al* 2003, Luo *et al* 2006, Reich *et al* 2006, Terrer *et al* 2018). Increased N availability typically results in increased above- and below-ground plant productivity, due to enhanced photosynthesis, resulting in increased soil C_{seq} through increased litter- and root-associated inputs, including labile C exudates (Lu *et al* 2011, Niu *et al* 2016, Yue *et al* 2016). High levels of forest C accumulation can be sustained if the ecosystem is able to continuously acquire enough N to meet their potential growth requirements, and/or if any available N can be utilized more effectively within their tissues, or by actively recycling it within the ecosystem (Luo *et al* 2004, Hungate *et al* 2006). Changes in the efficiency of N-use will inevitably cause changes in C:N ratio, with any increase in efficiency reflected in a higher C:N ratio. In a simulation study (Rastetter *et al* 1997) showed that the accumulation of N largely determined the long-term patterns of C gain by terrestrial ecosystems. If additional organic C inputs result in reduced N losses through increased N uptake, and/or there is enhanced biological fixation or increased atmospheric deposition, then progressive N limitation may not occur (Luo *et al* 2004, 2006). Whilst the increased decomposition of organic matter would not increase the N capital, it could also reduce N limitation by converting more N into inorganic forms that may be more usable by the vegetation. In addition, the stoichiometric relationship between C and N is flexible due to the transient stimulation of NPP and the increased absorption of available N by deeper plant roots, which may be crucial for C_{seq} during the early stages of forest succession (Comins and McMurtrie 1993, McMurtrie and Comins 1996, Rastetter *et al* 1997). However, the magnitude and way in which N availability regulates forest C_{seq} has not been well evaluated with observational data.

Elevated CO_2 experiments (e.g. Korner and Arnone 1992, Finzi *et al* 2006, 2007, Hungate *et al* 2006, 2013, Norby and Iversen 2006, Norby *et al* 2010, Sun *et al* 2018, Terrer *et al* 2018) provide invaluable evidence of the importance of understanding the significance of C–N coupling for C_{seq} by terrestrial ecosystems. However, it is still difficult to use these experiments to quantify the importance of N availability for long-term C_{seq} . In contrast, studies of forest succession over extended periods, including different stages of stand development, provides a way of assessing longer-term C and N dynamics and their relationship to C_{seq} (Johnson and Miyanishi 2008).

In the past decades, extensive research has been undertaken to examine how C–N stoichiometry varies along forest age gradients. For example, 115 years after the abandonment of agricultural areas in Rhode Island, USA, C:N ratios in different vegetation components and in mineral soils were reported to increase

with forest age (Hooker and Compton 2003). Yang *et al* (2014), in contrast, reported unchanged shoot C:N ratios but decreased soil C:N ratios, in a deciduous broadleaf forest over different stand ages on the eastern Tibetan Plateau. A synthesis of a number of studies showed generally increased root and shoot C:N ratios but unchanged soil and litter C:N ratios during forest stand development (Yang and Luo 2011). In general, due to large increases in C storage in vegetation, total ecosystem C storage increases with increasing forest age (Yang *et al* 2011), although soil C may show variable responses (Zak *et al* 1990, Pérez *et al* 2004, Rothstein *et al* 2004, O'Neill *et al* 2006, Gough *et al* 2007). Total ecosystem N amount may also increase with stand age through increased input from enhanced biological N fixation or atmospheric deposition, or through decreased losses due to reduced N leaching or gaseous emissions (Yang *et al* 2011). In many field studies, the total N content of vegetation and litter has been reported to increase with stand age (Hooker and Compton 2003, Noh *et al* 2010, Li *et al* 2013, Yang *et al* 2014, Jiang *et al* 2017). In contrast, the N in mineral soil has been reported to increase (Morris *et al* 2007, Sartori *et al* 2007) or decrease (Hooker and Compton 2003, Mao *et al* 2010) with stand age.

One way to examine any general patterns of C and N changes in secondary forests during forest succession is through the use of a meta-analysis approach that can quantitatively explore C–N interactions at time scales of decades or centuries (Borenstein *et al* 2011, Yang and Luo 2011, Yang *et al* 2011). This approach can also help to resolve interactions between different drivers from studies conducted in different regions or at smaller scales. In this study we synthesized published results from 135 individual studies aiming to resolve two major questions: (a) what is the temporal variation in C and N accumulation rates and C:N ratio changes in mineral soil, litter, and vegetation during forest succession? (b) what is the relationship between ecosystem C accumulation and changes in ecosystem N amount or variations in C:N ratio during forest succession? A major objective was to examine the hypothesis that variations in total ecosystem N amount is the dominant mechanism controlling long-term C_{seq} during forest succession.

2. Materials and methods

Data was collected from recently published literature from 1970 to 2018 reporting field investigations on dynamics of C and N stocks during forest succession (appendix figure S1, reference S1, table S1 (available online at stacks.iop.org/ERL/16/024055/mmedia)) and is an update of the dataset of Yang *et al* (2011). In order to maintain consistency with the study of Yang *et al* (2011), we used similar criteria (note S1)

for selecting and collating data on C and N dynamics. In total, 135 published papers were included in this study to compile a dataset including 2850 rows of observations and some basic information (geographic location, climatic information, forest type, stand age) for each study. The 12 variables of primary interest describe the C and N stocks (mg ha^{-1}) and their stoichiometric ratios in above- and below-ground vegetation, litter and soil pools. More information on data extraction and processing, as well as the conversion of the original soil C and N values from various depths to a standardized depth of 100 cm, are detailed in note S1 and Yang *et al* (2011).

We partitioned the C increment during stand development between that related to an altered stoichiometric flexibility and a changed total N amount, following the approach used by Hungate *et al* (2006) with more detailed description in note S1.

The increment in C mass of ecosystem component i during forest stand development induced by changes in C:N ratio ($\Delta C_{(C:N)i}$) was quantified using equation (1):

$$\Delta C_{(C:N)i} = \left[\left(\frac{C}{N} \right)_{(t+1)i} - \left(\frac{C}{N} \right)_{ti} \right] \times N_{ti} \quad (1)$$

where $\left(\frac{C}{N} \right)_{(t+1)i}$ and $\left(\frac{C}{N} \right)_{ti}$ are the C:N ratios of ecosystem component i at stand age $t + 1$ and the previous stand age t , and N_{ti} is the N amount of ecosystem i in a stand at age t .

The increment in C mass of ecosystem component i during forest stand development due to changes in N amount (ΔC_{TNi}) can be quantified using equation (2):

$$\Delta C_{TNi} = [N_{(t+1)i} - N_{ti}] \times \left(\frac{C}{N} \right)_{ti} \quad (2)$$

where $N_{(t+1)i}$ is the N amount of ecosystem component i in a stand at age $t + 1$.

Finally, the increment in C mass of ecosystem component i during forest stand development due to changes in both N amount and C:N ratios (an interactive component, $\Delta C_{\text{inter}i}$) can be expressed as

$$\Delta C_{\text{inter}i} = \left[\left(\frac{C}{N} \right)_{(t+1)i} - \left(\frac{C}{N} \right)_{ti} \right] \times [N_{(t+1)i} - N_{ti}]. \quad (3)$$

We applied this analysis to individual pools comprising above-ground vegetation, below-ground vegetation and litter using N mass and C:N data as described above. We used the sum of the C and N accumulation in these pools to assess the relative importance of the two pathways for C accumulation in vegetation.

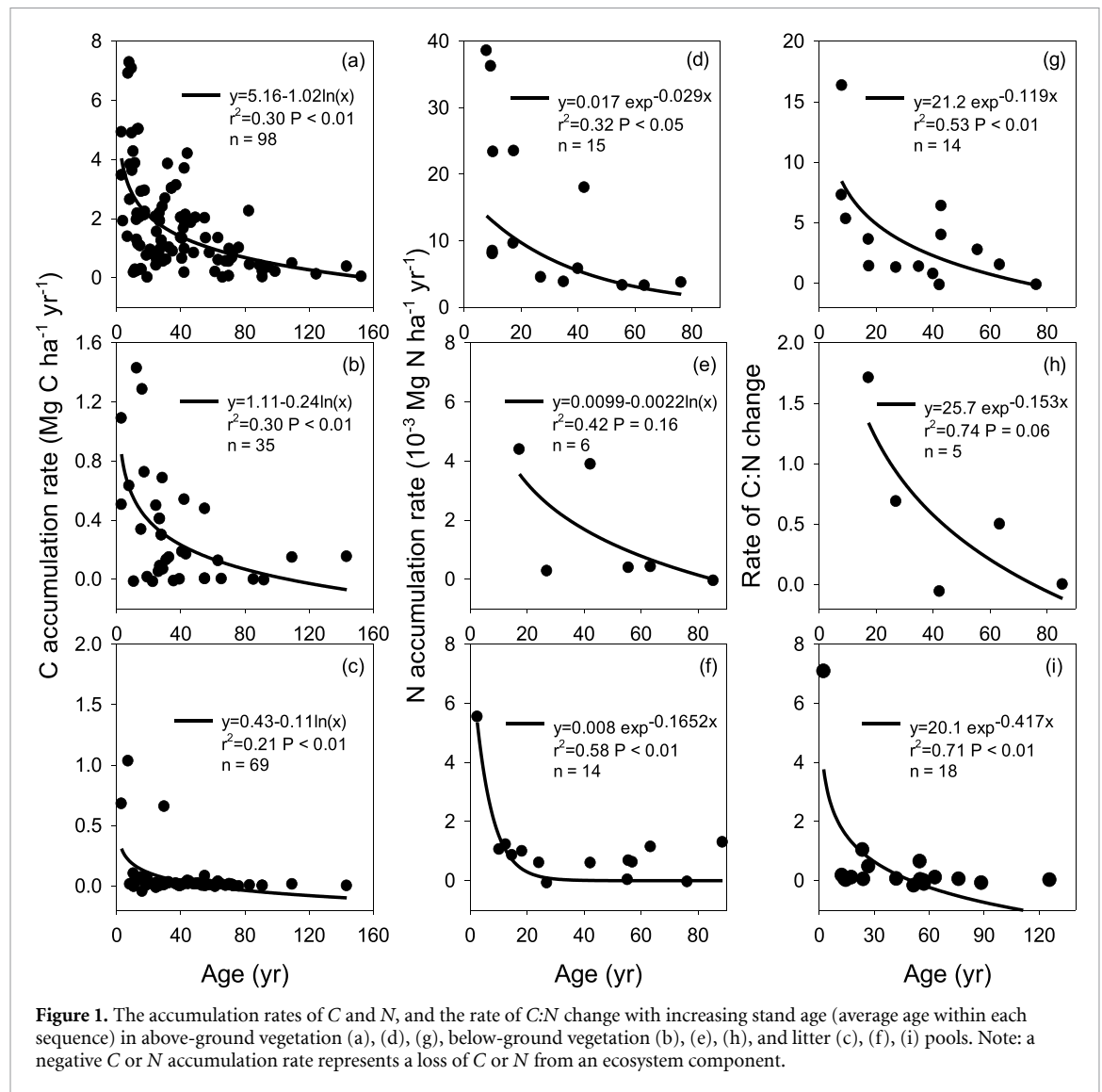
3. Results

3.1. Dynamics of C and N stocks and their stoichiometry along forest age gradient

The stocks of C and N in above- and below-ground components of the vegetation and litter increased during succession (figure S2), but no significant trends were noted in the C and N of soils (figure S3). The C and N accumulation rates (including rates of C:N change) in the three main ecosystem components exhibited similar decreasing patterns with stand age (figure 1). For instance, the C accumulation rate decreased markedly from ~ 4 to $\sim 2 \text{ mg C ha}^{-1} \text{ yr}^{-1}$ in the above-ground vegetation (figure 1(a)), from ~ 0.7 to $\sim 0.3 \text{ mg C ha}^{-1} \text{ yr}^{-1}$ in the below-ground vegetation (figure 1(b)), and from ~ 0.3 to $\sim 0.1 \text{ mg C ha}^{-1} \text{ yr}^{-1}$ in litter (figure 1(c)), during the first 30–40 years. These reductions then stabilized during the later stages of forest succession. The N accumulation rates (figures 1(d)–(f)) and the rates of change in the C:N ratio (figures 1(g)–(i)) in these vegetation components showed similar patterns to the C accumulation rates. The relationships with stand age are best described by either an exponential or a logarithm function. Consequently, C and N storage and C:N ratio in plant biomass or necromass pools increased along the age gradient and tended to level off during the later stages of forest succession (figure S2).

3.2. Contributions of plant N accumulation and stoichiometric flexibility to plant C storage

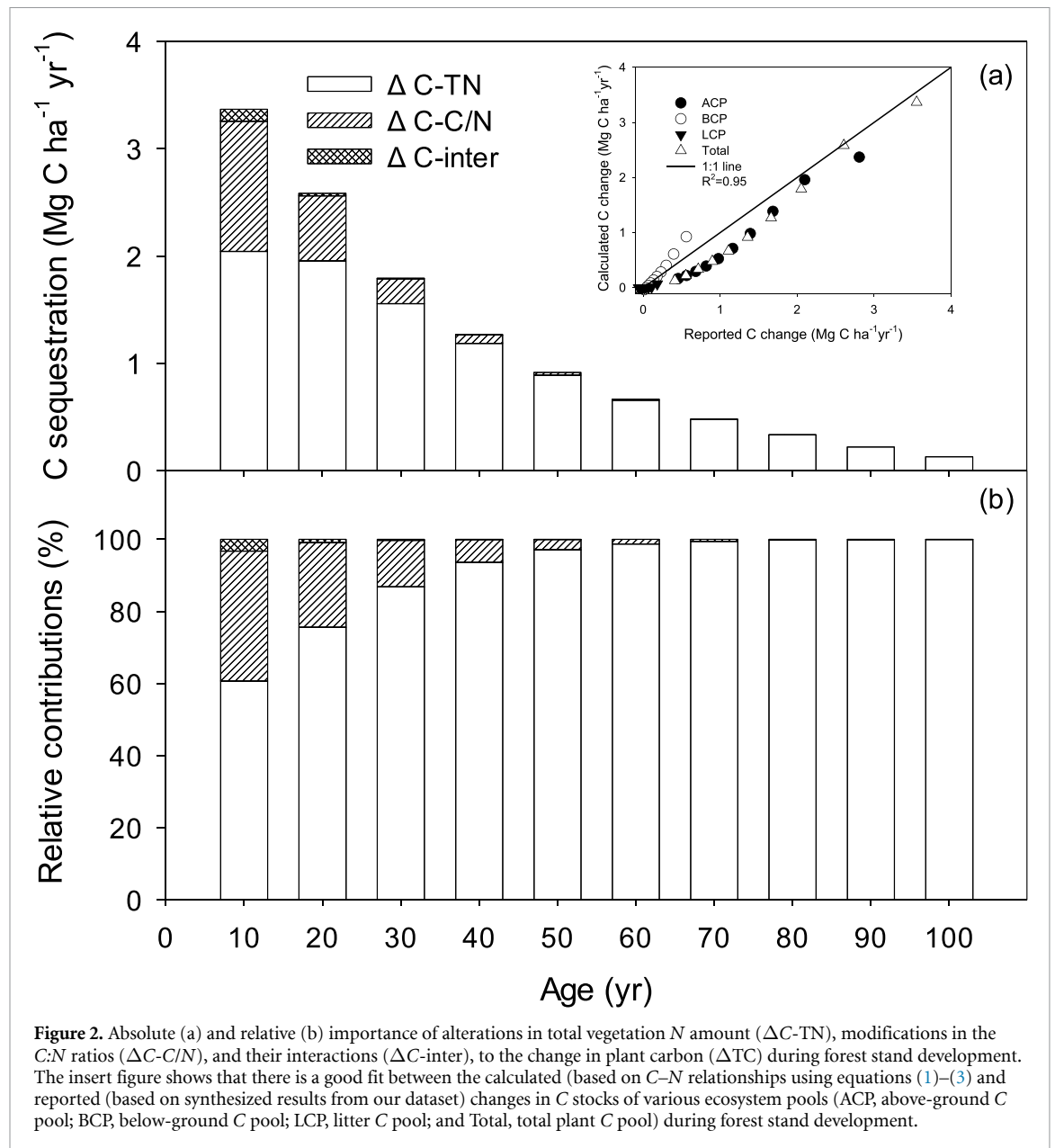
As shown in figure 2, contribution of alterations in total N amount and stoichiometric flexibility to ΔC across stand ages differed over time. In general, change in the amount of N was a more important contributor throughout all stages of forest stand development than any adjustments in C:N ratios, and its importance to C storage increased with forest age. In contrast, the importance of adjustments in C:N ratios to ΔC decreased with forest age (figure 2). More specifically, after 10 years of stand development, adjustments in C:N ratios contributed $\sim 40\%$ of ΔC ($\sim 1.2 \text{ mg ha}^{-1} \text{ yr}^{-1}$), whilst changes in the total amount of N contributed to $\sim 60\%$ of ΔC ($\sim 2.0 \text{ mg ha}^{-1} \text{ yr}^{-1}$). After 30–40 years of stand development, adjustments in C:N ratio contributed less than 10% of ΔC ($< 0.1 \text{ mg ha}^{-1} \text{ yr}^{-1}$), whilst changes in total N contributed over 90% of ΔC ($> 1.2 \text{ mg ha}^{-1} \text{ yr}^{-1}$). During an even later stage of stand development (i.e. after 60–70 years), the importance of adjustments in C:N ratio was negligible, whilst the importance of changes in the amount of N became almost the sole driver for ΔC (figure 2). The ΔC induced by the interactive combination of the two mechanisms (ΔC_{inter}) was of minor significance along the age gradient.



4. Discussion

The accumulation of ecosystem C during forest stand development was mainly associated with an increasing C accumulation in vegetation and litter (figure S2(a)) whilst C accumulation in mineral soil remained unchanged (figure S3(a)). Since N often limits forest productivity (Miller 1981, Magill *et al* 2000, Luo *et al* 2004, Yang *et al* 2011, Terrer *et al* 2018), long-term C_{seq} during forest stand development requires an input of N from soil or other sources or the more efficient use of N by the vegetation. The results of this analysis indicated that C accumulation during forest succession can be largely ascribed to changes in both total N amount and modification in C:N ratio (insert figure in figure 2). Increases in forest C stocks during the first 30 years of forest development was supported by a significant increase in N accumulation as well as by an expanded C:N ratio (figure 2), demonstrating a dependence on both N input and improvements in N use efficiency. At the latter stages of forest development

(30–40 years), the average rate of N accumulation was $\sim 4.0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, supporting over 90% of forest C_{seq} , which suggests that N availability overrides stoichiometric flexibility in determining long-term forest C_{seq} . This provides important insights into how forests use N during the development of stands (Takeuchi *et al* 2001, Crous and Ellsworth 2004, Sholtis *et al* 2004, Calfapietra *et al* 2005) and suggests that flexibility in the use of N is limited to early forest growth with C:N ratios remaining relatively stable after 30 years (Sistla and Schimel 2012). In contrast, C_{seq} by more mature forests is dependent on significant inputs of N. Of course, other factors, such as P availability, in particular, may play a more important role in tropical forests. In addition, there could be climate-related effects, as well as differences in C and N dynamics between needleleaf and broadleaf trees. However, the data is very limited, so that we could not carry out any further meaningful analysis related to climate zone or to differences between needleleaf and broadleaf trees and these require further investigation.



The unchanged C content in mineral soils suggest that C accumulation in vegetation and litter largely determined the extent of ecosystem C_{seq} during forest succession. Carbon accumulation in above-ground vegetation is largely associated with increases in stem/trunk height and girth. In the early stages of forest development, plant productivity is high due to high rates of photosynthesis and rapid increase in leaf area index, and then decreases until an equilibrium state is reached after canopy closure (Odum 1969, Gower *et al* 1996, Ryan *et al* 1997). Although a constant soil C content during forest succession may be somewhat surprising, this has also been reported in other studies (Gaudinski *et al* 2000, Yang *et al* 2011, Uri *et al* 2012). Whilst the increase in litter C (figure 1) may result in more C eventually being sequestered in soil (Chapin *et al* 2011), microbial decomposition is often observed to accelerate after reforestation and over the subsequent rotation because the higher

availability of soil-based and above-ground decomposable debris supports a larger and more diverse population of microfauna (Covington 1981, Zak *et al* 1990). Clearly, further consideration of the woody debris pool and the processes that contribute to its decomposition is important as this could represent an additional source of C and N. Whilst increases in tree mortality with succession will reduce litterfall and the contribution of C and N from vegetation into the soil pool (see Karelin *et al* 2020 for an example from the boreal region), a corresponding increase in woody debris could have the opposite effect and this requires quantification. At the same time NPP and net mineralization also decline, so both factors are likely to contribute to an overall decrease in C and N as forests age.

In addition, C outputs associated with the transfer of dissolved organic carbon (DOC) and particulate organic carbon (POC) to river networks might increase during forest succession (Ball *et al*

2010, Lajtha and Jones 2018). Thus, enhanced C input from plant litter could be offset by increased C losses through higher microbial activity and/or greater DOC or POC losses, resulting in a relatively unchanged soil C pool during forest succession. The variation in litter N accumulation seen in figure 1(f) may be a reflection of the differences in decomposition times found in studies like Kavadias *et al* (2001) where turnover times varies from 1 to 3 decades. Material entering the woody debris pool are shifted into an altered trajectory of slow C-release and slower N accumulation (Pastore *et al* 2019), however, the woody debris pool could not be assessed due to lack of data. Recent evidence also suggests that most C accumulated in soil is derived from labile compounds produced by roots (Sokol *et al* 2019) and this may decrease with stand development due to reductions in photosynthesis, or because of a reduced root mass per unit area due to self-thinning as forests age. This could counteract any increases in C due to litter incorporation. Clearly, more work is required in quantifying C inputs and outputs in forest soils.

It has been well recognized that the C:N ratio of above-ground vegetation and litter increases with stand age (Yang and Luo 2011), facilitating the accumulation of C without the need for additional N (Rastetter *et al* 1992, Luo *et al* 2004). Our results illustrate increased C:N ratio with forest age because C accumulates quicker than N during the first 20–30 years of stand development (figure 1). However, changes in C:N ratio stabilized during the latter stages (i.e. after 30 years) of forest succession (figure 1). Expansion of the C:N ratio in plant tissues may have been due to increases in structural woody tissues with larger C:N ratios than foliage (Ågren 2008, Janssens and Luyssaert 2009), which increases substantially during the early stages of stand development (Serner and Elser 2002, Ågren 2008). While the addition of more woody tissue would have little impact on the C:N ratio in older forests, where tree biomass is dominated by woody tissue with a high C:N ratio. The increased C:N ratio occurred mainly during the first 20–30 years of forest succession (figure 1) and is an important contributor to forest C_{seq} during this period, though the proportion of C supported by adjustments in the C:N ratio is still less than that supported by changes in the total N amount (figure 2). However, as trees age relatively more woody biomass is produced and canopy closure occurs, the influence of N-use efficiency on C_{seq} becomes greatly diminished and eventually becomes insignificant. Overall, changes in the total N amount outweighed the effects of any flexibility in the C:N ratio in determining forest C_{seq} during stand development and largely become the sole contributor after 30–40 years of forest succession (figure 2). Therefore, increases in N availability are the more typical mechanism supporting high forest C storage rates, particularly during the latter stages of

forest development. How long this lasts will depend on the availability of N to vegetation either through organic matter decomposition, the supply from other sources (e.g. atmospheric N deposition, biological N fixation), or the exploitation of formerly inaccessible resources through deeper or more extensive root systems. It is also important to recognize that by far the largest C store is in the above-ground vegetation where it is susceptible to the effects of biotic and abiotic factors as well as anthropogenic disturbances.

This work emphasizes that continuous increases in C storage are only sustainable when there is an external N input into the ecosystem (Luo *et al* 2004, 2006, Hungate *et al* 2006, Reich *et al* 2006, Yang *et al* 2014). In this study, the annual N accumulation, on average, was $\sim 5.6 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in above-ground vegetation, $\sim 1.9 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in below-ground vegetation, and $\sim 0.5 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in litter. Since the N content in mineral soil remained constant, the annual ecosystem N accumulation was approximately $8.0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, falling well within the range (0 to $120 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) reported previously (Binkley *et al* 2000). The accumulation of N during forest succession might, in many cases, be mainly due to external inputs via biological N fixation and atmospheric N deposition (Bormann 1993, Davidson *et al* 2007). Biological N fixation rates were reported to be 2.2, 4.9 and $2.9 \text{ g N m}^{-2} \text{ yr}^{-1}$, respectively, for boreal, temperate and tropical forests (Houlton *et al* 2008), whilst atmospheric N deposition rates were reported to vary from $< 0.2 \text{ g N m}^{-2} \text{ yr}^{-1}$ to $> 5 \text{ g N m}^{-2} \text{ yr}^{-1}$, depending on the region (Dentener *et al* 2006). Clearly, any inputs from N fixation will depend on the presence and activity of biological N fixers and it is evident that this is often an unlikely source of N as many secondary forests are largely devoid of N fixing species. Similarly, any increases in N through atmospheric N deposition are likely to be site-specific and regionally variable.

The availability of N during forest succession might also be influenced by legacies from past atmospheric N deposition, biological N fixation and previous land use history, including the afforestation of abandoned fertilized croplands (Kicklighter *et al* 2019). Also the exploitation of available soil N by roots from deeper soil reserves may increase with succession, resulting in the greater allocation of biomass to fine roots (Iversen 2010). A recent field investigation (Pastore *et al* 2019) suggests that the decomposition of litter and coarse woody debris can also lead to continuous N release, though this process is relatively slow. In addition, it has been suggested that the increased availability of N could be stimulated by rhizosphere interactions between plants and microorganisms (Phillips 2007, Frank and Groffman 2009). Subsequently, more N will become available for plant uptake in the mineral soil that could stimulate forest production over decades and centuries (Rastetter *et al* 1997). Nevertheless, a large

excess of N may down-regulate the fertilization effect of N on tree growth (Schulte-Uebbing and de Vries 2018) or even prove to be detrimental to further growth (Aber et al 1989). Like C , changes in the soil N stock are determined by both inputs (e.g. atmospheric N deposition, biological N fixation) and outputs (e.g. tree N uptake, gaseous or leaching loss of N). During forest development, the total N amount/capital in older forests is larger as they continue to accumulate N , although the accumulation rate declines with age due to a higher biomass than younger forests (Johnson 1992). If no external N can be supplied to trees, progressive N limitation may occur (Luo et al 2004) due to increasing N uptake and accumulation in forest standing biomass during forest development. In addition, gaseous losses of N (Benanti et al 2014) as well as dissolved organic N and particulate organic N leaching to river networks (Ball et al 2010) might also increase with stand age. The outputs of N might offset any increase in N as detailed earlier leading to unchanged soil N stocks, thus limited N availability in late succession could drive increasing rates of biological N fixation in forest ecosystems (Vitousek et al 2002, 2013, Zackrisson et al 2004). The N accumulation rates gradually decreased with forest age (figure 1), which may have been caused by more N being fixed in organic forms and a widened $C:N$ ratio in the litter, thereby reducing the available N , as has been reported in many field studies (Hooker and Compton 2003, Noh et al 2010, Li et al 2013, Yang et al 2014, Jiang et al 2017).

Due to the N stoichiometric relationship between vegetation and soil, it is essential that N is acquired from external resources in order to match the accumulation of ecosystem C (Hungate et al 2003). If an ecosystem is unable to accumulate enough N to support an increase in C_{seq} , its C storage capacity will be reduced and would not persist for extended periods of time (Hungate et al 2003, Luo et al 2004, Reich et al 2006, Wang et al 2020). As shown in this study, increases in both ecosystem N amount and $C:N$ ratios supported a positive C increment during different phases of forest succession. This study also showed that N accumulated continuously during forest succession; whilst the $C:N$ ratio increased dramatically in the early years of forest stand development they remained relatively constant during latter stages.

Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

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